

1 **A Highly Immunogenic Measles Virus-based Th1-biased COVID-19 Vaccine**

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11 **Keywords**

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13

14 **Abstract**

15 The COVID-19 pandemic is caused by severe acute respiratory syndrome coronavirus-2 (SARS-
16 CoV-2) and has spread world-wide with millions of cases and hundreds of thousands of deaths to
17 date. The gravity of the situation mandates accelerated efforts to identify safe and effective
18 vaccines. Here, we generated measles virus (MeV)-based vaccine candidates expressing the
19 SARS-CoV-2 spike glycoprotein (S). Insertion of the full-length S protein gene in two different MeV
20 genomic positions resulted in modulated S protein expression. The variant with lower S protein
21 expression levels was genetically stable and induced high levels of effective Th1-biased antibody
22 and T cell responses in mice after two immunizations. In addition to neutralizing IgG antibody
23 responses in a protective range, multifunctional CD8⁺ and CD4⁺ T cell responses with S protein-
24 specific killing activity were detected. These results are highly encouraging and support further
25 development of MeV-based COVID-19 vaccines.

26

27 **Author Contributions**

28 CH performed research, analyzed data, and wrote the paper; CS performed research and analyzed
29 data; AA performed research and analyzed data; AE performed research and analyzed data; SM
30 performed research, analyzed data, and wrote the paper; MH developed the bioinformatics pipeline
31 and analyzed data; BS contributed new reagents and concepts; MDM designed and supervised
32 research, analyzed data and wrote the paper; all authors read, corrected and approved the final
33 manuscript.

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35

36 **Significance Statement**

37 The COVID-19 pandemic has caused hundreds of thousands of deaths, yet. Therefore, effective
38 vaccine concepts are urgently needed. In search for such a concept, we have analysed a measles
39 virus-based vaccine candidate targeting SARS-CoV-2. Using this well known, safe vaccine
40 backbone, we demonstrate here induction of functional immune responses in both arms of adaptive
41 immunity with the desired immune bias. Therefore, occurrence of immunopathologies such as
42 antibody-dependent enhancement or enhanced respiratory disease is rather unlikely. Moreover,
43 the candidate still induces immunity against the measles, recognized as a looming second menace,
44 when countries are entrapped to stop routine vaccination campaigns in the face of COVID-19.
45 Thus, a bivalent measles-based COVID-19 vaccine could be the solution for two significant public
46 health threats.

47 **76 Introduction**

48
49 Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) belongs to *Coronaviridae* family
50 and emerged towards the end of 2019 as causative agent of pneumonia in the Hubei province in
51 China (1). The World Health Organisation named the disease Corona Virus Disease-2019 (COVID-
52 19), and officially declared the pandemic state on March 11, 2020. Human coronaviruses have
53 been known for decades as one of the causative agents of the common cold, but two previous
54 coronavirus outbreaks, caused by the severe acute respiratory syndrome virus (SARS-CoV-1) and
55 the Middle East respiratory syndrome virus (MERS-CoV), have demonstrated the remarkable
56 pathogenic potential of human beta coronaviruses. Around 10,000 people have been infected by
57 SARS and MERS, which has resulted in a death toll of about 1,500 patients, but the outbreaks
58 remained largely confined in terms of time or spread, respectively. In contrast, SARS-CoV-2
59 spreads effectively and at a rapid pace by direct transmission with an R_0 of at least 2 to 2.5 (2, 3).
60 Due to high transmissibility and extensive community spread, this novel coronavirus has already
61 caused over 12.1 million infections and over 550,000 deaths (as of 10 July 2020;
62 <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>), while world-wide shut-downs
63 of social life and economy to confine the spread of this respiratory virus are causing have
64 considerable impact.

65 After the emergence of SARS in 2002 and then MERS in 2012, vaccine development efforts have
66 been initiated, including the use of recombinant measles virus (MeV) vaccine as a platform concept
67 (4) to develop vector vaccine candidates against both agents and showed promising results.
68 Recombinant MeV vectors encoding the unmodified SARS-CoV Spike protein induced high titers
69 of neutralizing antibodies as well as IFN- γ T cell responses (5, 6) and conferred protection to
70 immunized animals upon pathogen challenge by lowering virus titers more than 100-fold (5). For
71 MERS, we have demonstrated that both, high titers of neutralizing antibodies as well as effective
72 and polyfunctional T cell responses, were induced in vaccinated animals (7, 8) and conferred
73 protection (7). Based on these data, a MeV-based MERS-vaccine candidate has been selected by
74 the Coalition for Epidemic Preparedness Initiative (CEPI) for further clinical development
75 (www.cepi.net/research_dev/our-portfolio).

76 Here, we explored the potential of recombinant MeV as vectors for the expression of the SARS-
77 CoV-2 spike protein (S) as successfully applied for the development of MERS- (7, 8) and SARS-
78 vaccine candidates (5, 6) as well as numerous other pathogens (4). The S glycoprotein was chosen
79 as antigen for its role as primary target of neutralizing antibodies (6, 7) and the exemplary capability
80 of MERS-CoV S protein to trigger strong cell-mediated immune responses when expressed by MeV
81 in our front-runner MERS vaccine candidate (7, 8). The SARS-CoV-2 S protein-encoding gene was
82 inserted into two different positions of the MeV genome to modulate antigen expression, and both

83 recombinant MeV were successfully rescued. The virus expressing lower S protein levels resulted
84 in stable amplification over at least 10 passages, while impairment of replication was insignificant.
85 Indeed, immunization of IFNAR^{-/-}-CD46Ge mice induced strong and functional humoral and cellular
86 immune responses directed against both MeV and SARS-CoV-2 S protein biased for Th1-type T
87 cell and antibody responses, illustrating the potential of MeV platform-based COVID-19 vaccine
88 candidates.

89

90

91 **Results**

92

93 **Generation and characterization of SARS-CoV-2-S by recombinant MeV_{vac2}**

94 Since the SARS-CoV and MERS-CoV spike proteins (S) have been shown to potently induce
95 humoral and cellular immune responses, the SARS-CoV-2 S protein was chosen as appropriate
96 antigen to be expressed by the recombinant MeV vaccine platform. A codon-optimized full-length
97 gene encoding SARS-CoV-2 S protein was cloned into two different additional transcription units
98 (ATUs) in the vaccine strain MeV_{vac2} genome, either downstream of the P (post P) or H (post H)
99 gene cassettes (Fig. 1A). Recombinant viruses were successfully generated and amplified up to
100 P10 in Vero cells with titers of up to 4×10⁷ TCID₅₀/ml. The stability of the viral genomes was
101 demonstrated via sequencing after RT-PCR of viruses in P2 or P10. In parallel to Sanger
102 sequencing of the ATU-region encompassing the SARS-CoV-2-S gene, the full genome was
103 sequenced using next generation sequencing with a coverage of 4 to 29,683 reads of each position
104 (Suppl. Fig. S1) in P2. Both methods revealed no mutations across the whole vaccine genomes
105 but a single A to G substitution on position 9 of the non-coding trailer region of the MeV_{vac2}-SARS2-
106 S(H) clone used for *in vivo* studies.

107 To verify SARS-CoV-2 S protein expression levels, Western blot analysis of Vero cells infected with
108 the MeV_{vac2}-SARS2-S was performed. The S protein expression was slightly attenuated when cells
109 were infected with viruses encoding the antigen in the ATU post-H compared to the post-P
110 constructs (Fig. 1B). However, there was less overall viral protein expression in cells infected with
111 post-P construct. Comparative growth kinetics with the vaccine viruses containing the SARS-CoV-
112 2 S gene and the MV_{vac2}-ATU(P) control virus revealed that the MeV_{vac2} encoding full-length SARS-
113 CoV-2 S gene in post-P position grew remarkably different to the control virus, with approximately
114 100-fold reduced maximal titers. In contrast, growth of MeV_{vac2}-SARS2-S(H) was much closer to
115 MV_{vac2}-ATU(P) with only a slight trend for lower titers (Fig. 1 C and D).

116 The impaired growth of MeV_{vac2}-SARS2-S(P) was accompanied by a hyper-fusogenic phenotype
117 (Fig. 1E, Suppl.Fig. S2A), which was also observed for the post-H vaccine candidate, but to a lesser
118 extent. Therefore, fusion activity was quantified and compared to the parental MV_{vac2}-ATU(P) as
119 well as the MV_{NSe}-GFP(N), which is known for its hyperfusogenic phenotype due to a V94M

120 substitution in the F₂ subunit of the MeV fusion protein (9). MV_{vac2}-ATU(P) induced fusion of
121 16.8±0.8 (mean±SD) Vero cells 30 h after infection. MeV_{vac2}-SARS2-S(P) revealed approximately
122 4-fold enhanced fusion activity (syncytia including 70±8 cells) while MeV_{vac2}-SARS2-S(H) just fused
123 41±6 cells, thereby representing an intermediate phenotype. However, fusion activity of the latter
124 became surpassed by MV_{NSe}-GFP(N) that fused 56±4 cells in 30 h under the same conditions
125 (Suppl. Fig. S2B).

126 To investigate if this increased fusion activity is due to SARS-CoV-2 S protein-mediated cell-to-cell
127 fusion, we expressed the SARS-CoV-2 S protein by transfection of the eukaryotic expression
128 plasmid pcDNA3.1-SARS2-S into SARS-CoV-2 receptor hACE2-negative 293T as well as into
129 receptor-positive Vero cells. Indeed, expression of SARS-CoV-2 S protein induced syncytia of
130 Vero, but not of 293T cells (Suppl. Fig. S3).

131 These data demonstrate that the hyperfusogenic phenotype of the SARS-CoV-2 S-encoding MeV
132 is linked to expression of a fusion-active form of the SARS-CoV-2 S protein, indicating that cells
133 infected by the vaccine candidates express a functional S protein. Thus, cloning and rescue of
134 MeVs expressing correctly folded SARS-CoV-2 S was achieved successfully. Since higher S
135 protein expression levels impaired viral replication, MeV_{vac2}-SARS2-S(H) was chosen for further
136 characterization *in vivo*.

137

138 **MeV_{vac2}-SARS2-S(H) induces neutralizing antibodies against MeV and SARS-CoV-2**

139 To test the efficacy of MeV_{vac2}-SARS2-S(H) *in vivo*, genetically modified IFNAR^{-/-}-CD46Ge mice
140 were used, since they are the prime small animal model for analysis of MeV-derived vaccines (10).
141 Groups of 4 - 6 animals were immunized via the intraperitoneal (i.p.) route on days 0 and 28 with
142 1×10⁵ TCID₅₀ of MeV_{vac2}-SARS2-S(H) or empty MV_{vac2}-ATU(P) as a control. As positive control,
143 recombinant SARS-CoV-2 S protein adjuvanted with aluminum hydroxide gel (Alum) was injected
144 subcutaneously, and medium-inoculated mice served as mock controls. 21 days after the second
145 immunization, sera of immunized mice were analyzed in comparison to pre-bleed and post-prime
146 immunization sera by ELISA on antigen-coated plates for total IgG antibodies binding to MeV bulk
147 antigens (Fig. 2G-I) or SARS-CoV-2-S protein (Fig. 2A-C). Sera of mice vaccinated with MeV_{vac2}-
148 SARS2-S(H) contained IgG antibodies that bound to SARS-CoV-2-S protein (Fig. 2B and C),
149 whereas no antibodies were found in mice before vaccination (Fig. 2A), or in MeV or mock-
150 immunized control mice. Moreover, final sera of mice vaccinated with any recombinant MeV had
151 IgG in the serum binding to MeV bulk antigens, indicating at least one successful vaccination with
152 MeVs and general vector immunogenicity (Fig. 2G-I). The control S protein vaccine did induce
153 higher levels of S protein-binding IgG than MeV_{vac2}-SARS2-S(H).

154 We next determined the neutralizing antibody responses against SARS-CoV-2 (Fig. 2D-F) or MeV
155 (Fig. 2J-L). Most mice immunized with recombinant MeV, including those receiving the control virus,

156 had developed MeV neutralizing antibody titers (VNT) after the first immunization (Fig. 2K).
157 However, one mouse of the MeV_{vac2}-SARS2-S(H) cohort initially reacted only weakly and another
158 mouse not at all, reflecting individual differences in response to immunization. All animals had
159 developed neutralizing antibodies after the second immunization, and a 3-fold increase was
160 observed upon the second immunization (257 to 800 VNT, Fig. 2K, L). Neutralizing antibodies
161 against SARS-CoV-2 were detected in mice vaccinated with MeV_{vac2}-SARS2-S(H) after the second
162 immunization, and reached a titer of 15 to 80 in three out of 6 mice (Fig. 2F). These titers were in
163 the range of human convalescent sera tested in parallel (VNT 10 to 60; Fig. 2M). No VNTs against
164 MeV or SARS-CoV-2 were detected in control mice inoculated with medium alone. Interestingly,
165 the alum-adjuvanted recombinant S protein did not induce any neutralizing antibodies despite
166 higher binding IgG levels in ELISA, indicating that these antibodies bind to other epitopes of S or
167 with lower affinity than those induced by the MeV-based vaccine candidate. In summary, the SARS-
168 CoV-2-S protein-expressing MeV elicited robust neutralizing antibody responses against MeV and
169 SARS-CoV-2.

170

171 **Splenocytes of animals vaccinated with MeV_{vac2}-SARS2-S(H) react to SARS-CoV-2 S protein-** 172 **specific stimulation**

173 To assess the ability of MeV_{vac2}-SARS2-S(H) to induce SARS-CoV-2-specific cellular immune
174 responses, splenocytes of vaccinated animals were analyzed for antigen-specific IFN- γ secretion
175 by ELISpot assay. Towards this, antigen-specific T cells were re-stimulated by co-cultivation with
176 the syngeneic murine DC cell lines JAWSII or DC2.4 stably expressing the SARS-CoV-2 S protein.
177 For JAWSII cells, bulk cultures of transduced cells were obtained by flow cytometric sorting. For
178 DC2.4 cells, single cell clones were generated by limiting dilution of sorted respective of bulks
179 cultures. Antigen expression by transduced DCs was verified by Western Blot analysis (data not
180 shown).

181 ELISpot assays using splenocytes of vaccinated animals in co-culture with DC2.4-SARS2-S cells
182 revealed more than 1,400 IFN- γ secreting cells per 1×10^6 splenocytes after immunization with
183 MeV_{vac2}-SARS2-S(H), respectively (Fig. 3). In contrast, co-culture with splenocytes of control mice
184 resulted in a background response of less than 50 IFN- γ producing cells per 1×10^6 splenocytes. As
185 expected, re-stimulation of T cells by DC2.4 presenting no exogenous antigen revealed only
186 reactivity in the range of background (Fig. 3). To rule out clonal or cell line-associated artifacts,
187 antigen-specific IFN- γ secretion by splenocytes of MeV_{vac2}-SARS2-S(H) vaccinated mice was
188 confirmed by stimulation with transgenic JAWSII-SARS2-S bulk cells. These cells also stimulated
189 in excess of 1,400 IFN- γ secreting cells per 1×10^6 splenocytes in animals receiving the recombinant
190 SARS-CoV-2 vaccines, whereas only slight background stimulation was observed by the
191 respective controls. The differences between MeV control and MeV_{vac2}-SARS2-S vaccinated mice

192 were statistically significant for both cell lines. Mice vaccinated with Alum-adjuvanted S protein
193 showed no specific reactivity in IFN- γ ELISpot.

194 Cellular immune responses upon stimulation with MeV bulk antigens were detected in animals that
195 had been vaccinated with any recombinant MeV virus, as expected. While MeV bulk antigens
196 stimulated only about 300 to 700 IFN- γ secreting cells per 1×10^6 splenocytes of MV_{vac2}-ATU(P)
197 vaccinated animals, but 400 to 1,400 IFN- γ secreting cells per 1×10^6 splenocytes of MeV_{vac2}-
198 SARS2-S(H) vaccinated animals. However, this trend was not statistically significant. Splenocytes
199 of all animals revealed a similar basic reactivity to unspecific T cell stimulation, as confirmed by
200 numbers of IFN- γ secreting cells upon ConA treatment at the limit of detection. Remarkably,
201 stimulation of splenocytes by DC2.4 expressing SARS-CoV-2-S resulted in at least similar or even
202 higher numbers of IFN- γ^+ cells than after stimulation by MeV bulk antigens, indicating an extremely
203 robust induction of cellular immunity against this antigen. Taken together, these data show that
204 MeV_{vac2}-SARS2-S(H) not only induces humoral, but also strong SARS-CoV-2-S protein-specific
205 cellular immune responses.

206

207 **SARS-CoV-2 S-reactive T cells are multifunctional**

208 To gain more detailed insights in the quality of the observed T cell responses, we further
209 characterized the responsive T cell populations by flow cytometry, determining the expression of
210 IFN- γ , TNF- α and IL-2 in CD8 $^+$ and CD4 $^+$ positive CD3 $^+$ T cells upon re-stimulation with SARS-
211 CoV-2 S-presenting DC2.4-SARS2-S cells by intracellular cytokine staining (ICS). As a positive
212 stimulus for T cell activation, tetradecanoylphorbol-acetate and ionomycin (TPA/Iono) were used.
213 Exocytosis of cytokines was blocked by addition of brefeldin A (10 μ g/mL) during stimulation. Cells
214 were permeabilized, labelled, and fixed for flow cytometry. The gating strategy excluded duplicates
215 (Suppl. Fig. S4, top row, middle panel), selected for living cells (Suppl. Fig. S4, top row, right panel),
216 and separated CD8 $^+$ and CD4 $^+$ T cells on CD3 $^+$ cell populations (Suppl. Fig. S4, 2nd row). Selected
217 T cells were then analyzed for their expression of IFN- γ , TNF- α , or IL-2, double- (Suppl. Fig. S4,
218 3rd row), or triple-positive cells (Suppl. Fig. S4, bottom row) as exemplarily shown for CD4 $^+$ T cells
219 after re-stimulation with TPA and ionomycin (Suppl. Fig. S4).

220 Vaccination with MeV_{vac2}-SARS2-S(H) induced a significant amount of SARS-CoV-2 S-specific
221 CD8 $^+$ T cells expressing either IFN- γ (Fig. 4B, left panel), IL-2 (Fig. 4B, middle panel) or TNF- α
222 (Fig. 4B, right panel), with means between 0.1% and 0.5% of positive cells for each of these
223 cytokines. Among those, a significant fraction of cells proved to be multifunctional, with a mean of
224 49% of the reactive CD8 $^+$ cells expressing two cytokines or 13% of responsive CD8 $^+$ cells being
225 positive for TNF- α , IL-2 and IFN- γ (Fig. 4C). A much lower portion of responsive CD4 $^+$ T cells was
226 observed, varying between 0.01% to 0.07% of CD4 $^+$ T cells. Among the responsive CD4 $^+$ cells,

227 46% expressed two cytokines and 10% were positive TNF- α , IL-2 and IFN- γ . Moreover,
228 vaccination induced a significant fraction of vector-specific CD4⁺ T cells expressing IFN- γ (Fig. 4A,
229 left panel), IL-2 (Fig. 4A, middle panel) or TNF- α (Fig. 4A, right panel) upon re-stimulation with MeV
230 bulk antigen. Among those, multifunctional CD4⁺ T cells expressing two or all three cytokines were
231 induced with a mean of about 22% and 6% poly-reactive T cells (Fig. 4C), respectively. To
232 conclude, vaccination with MeV_{vac2}-SARS2-S(H) induces not only IFN- γ , TNF- α , or IL-2 expressing
233 T cells directed against SARS-CoV-2 and MeV, but also a significant fraction of multifunctional
234 cytotoxic T cells specific for SARS-CoV-2 S and CD4⁺ T cells specific for MeV antigens, illustrating
235 that a broad and robust SARS-CoV-2-specific immune response is induced by vaccination with
236 MeV_{vac2}-SARS2-S(H).

237

238 **MeV_{vac2}-SARS2-S(H) induced antigen-specific CD8⁺ and CD4⁺ T cells respond with**
239 **proliferation.**

240 While ELISpot and ICS analyses revealed antigen-specific cytokine secretion by vaccinated mice'
241 T cells, we next aimed at detecting antigen-specific CD8⁺ cytotoxic T lymphocytes (CTLs) which
242 would be important for clearance of virus infected cell and CD4⁺ T helper cells. For that purpose,
243 proliferation of CD8⁺ and CD4⁺ T cells upon stimulation with SARS-CoV-2-S was analyzed 3 weeks
244 after the boost via a flow cytometry. Splenocytes of mice were isolated 21 days after the boost, and
245 DC2.4-SARS2-S cells were used for re-stimulation of T cells. The splenocytes were labelled with
246 CFSE and subsequently co-cultured with DC2.4-SARS2-S cells or, as a control, with parental
247 DC2.4 cells for 6 days and finally stained for CD3, CD4, and CD8 before being analyzed for
248 proliferation, detectable by the dilution of the CFSE stain due to cell division.

249 T cells of mice vaccinated with MeV_{vac2}-SARS2-S(H) revealed an increase in the population of
250 CD3⁺CD4⁺CFSE^{low} and CD3⁺CD8⁺CFSE^{low} cells after re-stimulation with DC2.4-SARS2-S cells
251 compared to re-stimulation with parental DC2.4 without SARS-CoV-2 S antigen (Fig. 5). In contrast,
252 T cells of control mice did not reveal this pattern, but the CFSE^{low} population remained rather
253 constant. The prominent increase in CD3⁺CD8⁺CFSE^{low} cells, which was significant for MeV_{vac2}-
254 SARS2-S(H) vaccinated mice, indicates that CD3⁺CD8⁺ CTLs and CD3⁺CD4⁺ T helper
255 lymphocytes specific for SARS-CoV-2 S have proliferated upon stimulation. Thus, SARS-CoV-2-
256 specific cytotoxic memory T cells are induced in mice after vaccination with MeV_{vac2}-SARS2-S(H).

257

258 **Induced T cells reveal antigen-specific cytotoxicity.**

259 To demonstrate the effector ability of induced cytotoxic T lymphocytes (CTLs), a killing assay was
260 performed to directly analyze antigen-specific cytotoxicity (Fig. 6). Splenocytes of immunized mice
261 isolated 21 days post boost vaccination were co-cultured with DC2.4-SARS2-S or parental DC2.4
262 cells for 6 days to re-stimulate antigen-specific T cells. When these re-stimulated T cells were co-

263 incubated with a defined mixture of EL-4_{green}-SARS2-S target and EL-4_{red} control cells (ratio
264 approximately 1:1), only T cells from MeV_{vac2}-SARS2-S(H) vaccinated mice significantly shifted the
265 ratio of live SARS-CoV-2 S protein-expressing target cells to control cells in a dose-dependent
266 manner (Fig. 6B). This antigen-dependent killing was also dependent on re-stimulation with DC2.4-
267 SARS2-S cells, since unstimulated T cells did not significantly shift the ratios of target to non-target
268 cells (Fig. 6A).

269 These results indicate that CTLs isolated from MeV_{vac2}-SARS2-S(H)-vaccinated mice are capable
270 of lysing cells expressing SARS-CoV-2 S. Neither splenocytes of control mice re-stimulated with
271 DC2.4-SARS2-S nor splenocytes of SARS-CoV-2-S vaccinated mice re-stimulated with control
272 DC2.4 cells showed such an antigen-specific killing activity, demonstrating that MeV_{vac2}-SARS2-
273 S(H) induces fully functional antigen-specific CD8⁺ CTLs.

274 **Induced immunity is skewed towards Th1-biased responses.**

275 While the functionality of both humoral and cellular anti-SARS-CoV-2 immune responses elicited
276 by MeV_{vac2}-SARS2-S(H) is reassuring, the SARS-CoV-2 vaccine development has to proceed with
277 some caution because of the potential risk of immunopathogenesis observed in some animal
278 models, such as antibody-dependent enhancement (ADE) and enhanced respiratory disease
279 (ERD) which seem to correlate with a Th2-biased immune response. Since in mice IgG1 is a marker
280 for Th2-bias and risk of ADE development, whereas IgG2a antibodies indicate a favorable Th1-
281 bias, IgG subtype-specific ELISA were performed with the sera collected at different time points.
282 Animals vaccinated with alum-adjuvanted SARS-CoV-2 S protein, a vaccine concept known for its
283 Th2-bias (11, 12), developed high levels of S protein-specific IgG1 antibodies, whereas few S-
284 specific IgG2a antibodies were detected (Fig. 7A). In comparison, MeV_{vac2}-SARS2-S(H) induced
285 100-fold less IgG1 antibodies, but at least 10-fold higher IgG2a levels (Fig. 7A), indicating a
286 favorable Th1-bias in animals immunized with the MeV-derived vaccine candidate.

287 These findings were confirmed by multiplex cytokine analysis of the cytokine profile in the
288 supernatants of splenocytes from vaccinated animals, which were re-stimulated using DC2.4 or
289 DC2.4-SARS2-S cells. All splenocytes revealed secretion of all cytokines after stimulation with
290 ConA demonstrating general reactivity of cells and assay (data not shown). Most likely due to the
291 low number of S-reactive T cells in animals that had been vaccinated with recombinant SARS2-S
292 protein and Alum, no or minimal, constant cytokine levels were measurable in the supernatants of
293 re-stimulated splenocytes (Fig. 7B). In contrast, splenocytes of animals immunized with MeV_{vac2}-
294 SARS2-S(H) reacted specifically with the secretion of IFN- γ , TNF- α , and IL-2 upon re-stimulation
295 by DC2.4-SARS2-S (Fig. 7B, top row), in accordance with ELISpot and ICS data. However, we
296 could observe no or minimal up-regulation of IL-4, IL-5, IL-13, or IL-10, which would have been

297 indicative for a Th2-biased response (Fig. 7B, middle row). Also IL-17a, or IL-6 indicative of a Th17
298 or general inflammatory response showed minimal changes (Fig. 7 B, bottom row).

299 Thus, both humoral and cellular responses reveal a Th1-biased immunity induced by MeV_{vac2}-
300 SARS2-S(H), which indicates a relatively low risk for putatively Th2-mediated immunopathologies.

301
302

303 **Discussion**

304
305 In this study, we aimed to analyze the efficacy of MeV-derived vaccine candidates encoding the
306 Spike glycoprotein S of SARS-CoV-2 to induce functional immune responses to protect against
307 COVID-19. We show that MeV_{vac2}-SARS2-S(H) replicated comparably to MeV vaccine strain
308 viruses and was genetically stable over extended passaging. Upon vaccination of mice, it induced
309 robust humoral immune responses of the IgG2a subtype directed against the SARS-CoV-2 spike
310 glycoprotein S with neutralizing activity in a range already shown to be protective by others. In
311 addition, considerable amounts of SARS-CoV-2 S-specific CD4⁺ and CD8⁺ T cells were induced,
312 the major fraction of which were secreting two or even all three cytokines when analysing for IFN- γ ,
313 TNF- α , or IL-2 upon antigen-specific re-stimulation. These T cells proliferated and specifically
314 depleted antigen-positive target cells in a mixed population. Importantly, all responses were skewed
315 toward Th1-biased immunity. In parallel, the capacity to induce measles-specific immune reactivity
316 remained conserved.

317 This effective MeV Moraten strain-derived recombinant vaccine MeV_{vac2}-SARS2-S(H) is a live-
318 attenuated vaccine that encodes the full-length, functional version of the SARS-CoV-2 Spike
319 protein as main target for functional antibodies, but also for induction of T cell responses. Vero cells
320 revealed homogenous expression of the SARS-CoV-2 S antigen by Western Blot analyses and
321 positive immunostaining of syncytia after infection by MeV_{vac2}-SARS2-S(H). Stable antigen
322 expression is a prerequisite for the immune system to encounter the specific antigen to mount
323 robust immune responses and for industrial production of a vaccine. Indeed, IFNAR^{-/-}-CD46Ge
324 mice vaccinated with MeV_{vac2}-SARS2-S(H) in a prime-boost protocol showed uniform induction of
325 antibodies directed against MeV bulk antigens or SARS-CoV-2 S, which had considerable
326 neutralizing activity against both pathogens. We observe antibody responses in these animals at a
327 level that correlate with protection in mouse challenge models (13), as well as with neutralizing
328 activity we found in the serum of 4 reconvalescent human patients. These responses were triggered
329 even though the knock-out of the type I interferon receptor, which is necessary to allow propagation
330 of MeV in mice (10, 14). This knock-out usually should impair the induction of especially humoral
331 immune responses (15). This highlights the remarkable immunogenicity of the MeV vaccine
332 platform technology that also works in this model with partially impaired immune responses.

333 However, why did not all immunized animals develop neutralizing activity detectable in our assay?
334 Firstly, determination of the VNT relying on 100% pathogen neutralization is obviously a rather
335 harsh assay in the context of SARS-CoV-2, as evidenced by the modest VNT titers published so
336 far, in general, and absence of VNT in the S+Alum vaccinated group despite high amounts of S
337 binding antibodies. This means that just detectable VNT already indicates considerable neutralizing
338 activity. Secondly, we realized that two of the three animals which did not show a VNT >10 have
339 not responded well to the prime vaccination, at all. These animals developed none or only a minor
340 VNT against MeV after the first vaccination. This observation is rather unusual, and argues for
341 technical issues during the first vaccination in these two animals. Since none of the animals showed
342 VNT against SARS-CoV-2 after one vaccination with the vaccine, it is tempting to speculate that a
343 prime-boost-protocol is associated in this animal model with maturation of antibodies to generate
344 better neutralizing responses. On the other hand, all animals including the two improperly
345 immunized ones revealed significant, multi-functional T cell responses against SARS-CoV-2 S,
346 which were still recordable three weeks after the second vaccination, when we already expect
347 constriction of antigen-specific T cell effector populations. These data suggest that anti-S antibody
348 responses mature after repeated vaccination, but on the other hand that a one shot vaccination
349 regime will already induce especially functional memory T cell immune responses, the protective
350 efficacy of which as well as their duration has to be demonstrated in future challenge experiments.
351 In any case, we have observed with other foreign viral antigens that these T cell responses can be
352 detected in mice more than 2 years after vaccination (Hörner & Fiedler et al., unpublished data).
353 This observation is in accordance with the stability of anti-measles immunity (16) also after pediatric
354 vaccination (17) and might be a specific advantage of the measles vaccine platform technology.
355 Also extended passaging of the vaccine candidate did not result in changes of the vaccine as
356 revealed by sequencing of the virus after 10 passages starting with a low MOI. This genetic stability
357 indicates that the slight impairment seen in multi-step growth curves when compared to a vaccine-
358 strain MeV is not critical for the vaccine's amplification and therefore crucial for product safety. In
359 accordance with its genetic stability, the minor enhancement of fusion activity can also be regarded
360 as non-critical, especially with a view on the fusion activity of MeV used in clinical trials for treatment
361 of tumors. These so called oncolytic MeV have been used in 15 phase 1 and phase 2 clinical trials,
362 so far. Thereby, advanced-stage tumor patients suffering from different tumor entities have been
363 treated. Despite constituting in principle a vulnerable patient collective, application of high doses of
364 non-targeted, fusion-active MeV (up to 1×10^{11} TCID₅₀) (18) systemically or for example directly into
365 the patients' brains (19) was accompanied by an acceptable safety profile (20). Therefore, the
366 enhancement of fusion activity cannot be expected to be crucial for product safety, while the
367 attenuation of vaccine-strain MeV is multifactorial, anyway, and not just a matter of cell entry
368 tropism and mechanism (21). Likewise, the clinical phase 1 and 2 trials using the MeV vector

369 platform for the generation of bivalent vaccines, which induce immunity against CHIKV (22, 23),
370 have revealed an extremely beneficial safety profile of this recombinant vaccine concept also in
371 human patients, while signs of efficacy became evident.

372 In any case, generation of MeV-derived COVID-19 vaccines encoding a less fusion-active variant
373 of the SARS-CoV-2 Spike glycoprotein might be beneficial to enhance titers of the vaccine virus.
374 In the meantime, stabilized S variants have become available that have attenuated or no cell-cell
375 fusion activity. One variant has a deletion of the multi-basic cleavage motif for furin-like proteases
376 at the S1/S2 boundary that facilitates pre-activation of S (24). A second variant has proline
377 substitutions at residues 986 and 987, which are stabilizing a pre-fusion conformation of S (25).
378 Vaccine candidates encoding S with one of these motifs or a combination thereof in a soluble
379 version as already done for DNA vaccines (26) are under development. These have to show an at
380 least comparable capacity to induce neutralizing antibody responses also in the context of MeV
381 infection, which might be dependent on the respective conformation of the antigen that is expressed
382 by vaccine virus-infected cells *in situ*.

383 The induction of the “right” antibodies and T cell responses is especially crucial with a view on
384 potential complications that can be observed when coronavirus encounter “wrong” immune
385 responses that can give rise to immunopathologies after infection. In some infected cats, infection
386 with feline coronavirus causes feline infectious peritonitis, a deadly disease characterized by viral
387 infection of macrophages during the acute phase. Interestingly, the switch of pathology after
388 infection from a rather moderate pathogenesis into an acute, devastating disease can be triggered
389 by vaccination of persistently infected cats and has been attributed to the induction of antibodies
390 that mediate enhancement of the disease, a process called antibody-dependent enhancement
391 (ADE). During COVID-19, ADE might be the cause of the severe cases currently observed. Some
392 case reports indicate that severe disease appeared more frequently in patients with high SARS-
393 CoV-2 immunoglobulin G (IgG) levels (27). ADE has been most prominent for dengue virus (DENV)
394 infections, especially in secondary infections with a different DENV serotype where enhancement
395 of disease correlated with the induction of non-neutralizing Abs that can mediate an efficient uptake
396 of the virus in FcR-positive cells such as macrophages and other immune cells (28). Moreover,
397 other immune-related adverse events were described for SARS- and MERS-CoV. When animals
398 were immunized with vaccines that pre-dominantly induce Th2-biased T-helper cell responses,
399 vaccinated mice revealed significantly reduced virus loads after challenge, but also an eosinophilic
400 infiltrate into the lungs accompanied by pathological changes of the lung tissue, so called enhanced
401 respiratory disease (ERD) (29). Such immunopathologies upon CoV infection are a major concern
402 for diseases pathology and especially vaccine development. Thus, Th2-biased immune responses
403 as triggered by alum-adjuvanted whole inactivated virus particles or recombinant proteins should
404 be avoided.

405 Interestingly, the live-attenuated MeV vaccine is known for a balanced Th1/Th2-bias of induced
406 immune responses with a bias for Th1 responses at least during the acute phase after vaccination
407 (30). In theory, this should also apply for immune responses induced against all antigens presented
408 during a MeV vaccine virus infection including foreign antigen(s) additionally expressed when MeV
409 is used as vaccine platform. Indeed, our analyses provide evidence that the bias of the immune
410 responses is in favour of Th1 responses, as revealed by the inverted IgG1/IgG2a subtype ratio of
411 antibodies induced against SARS-CoV-2 S by MeV_{vac2}-SARS2-S(H) compared to the animals
412 immunized with alum-adjuvanted recombinant S protein. Moreover, the cytokine profile of
413 splenocyte cultures of immunized mice after re-stimulation of S-specific T cells reveals a respective
414 preferable Th1 bias. Since SARS-CoV-2 and SARS-CoV use the same primary attachment
415 receptor for cell entry, hACE2, and selected hACE2-transgenic mice show differential pathology
416 after inoculation with SARS-CoV-2 (13, 31), studying the impact of the Th1-biased MeV-based
417 immunization in hACE2-transgenic mice during challenge with SARS-CoV-2 will be a matter of
418 future studies. In any case, our data suggest that MeV-derived COVID-19 vaccines have a low
419 likelihood to trigger immunopathogenesis. Another animal model for COVID-19, golden Syrian
420 hamsters, could be an alternative for future challenge studies. This animal model is susceptible for
421 SARS-CoV-2 infection, reveals a moderate, but clearly distinguishable pathology, and shows air-
422 borne transmissibility from infected to naïve animals (32, 33). Therefore, this animal model
423 accurately reflects at least some aspects of the course of human disease and should be valuable
424 for assessment of the protective efficacy of COVID-19 vaccines.

425 In conclusion, the bivalent MeV/SARS-CoV-2 vaccine candidate has a number of desirable
426 properties with respect to its immunogenicity against SARS-CoV-2. Furthermore, the concurrent
427 induction of anti-MeV immunity would allow its use in the context of routine measles immunization
428 schedules. Such a MeV-based COVID-19 vaccine could be included in the currently applied MMR
429 (measles, mumps, rubella) vaccine, providing additional protection against SARS-CoV-2. While
430 controversially discussed to which extent, children do become infected and shed the virus, despite
431 them rarely being severely affected. In any case, preventing infection or virus shedding from
432 vaccinated children can also help to contain the disease and protect vulnerable patient groups.
433 Moreover, the capacity to produce large amounts of vaccine doses would be available more or less
434 instantly from routine measles vaccine production, but at no impairment of production of other
435 necessary vaccines, since the measles vaccine property is preserved in the proposed vaccine
436 candidate. Especially since vaccination against the measles should not be impaired also during the
437 COVID-19 epidemic, this is a considerable advantage. Otherwise, parallel epidemics with another,
438 even more contagious respiratory virus are looming when vaccination programs against the
439 measles are stopped in favour of COVID-19 vaccination programs. Therefore, MeV_{vac2}-SARS2-
440 S(H) is a promising vaccine candidate that warrants further investigation.

441

442 **Materials and Methods**

443

444 **Cells**

445 Vero (African green monkey kidney) (ATCC# CCL-81), Vero clone E6 (ATCC# CRL-1586), 293T
446 (ATCC CRL-3216) and EL-4 (ATCC TIB-39) cell lines were purchased from ATCC (Manassas, VA,
447 USA) and cultured in Dulbecco's modified Eagle's medium (DMEM, Biowest, Nuaille, France)
448 supplemented with 10% fetal bovine serum (FBS; Biochrom, Berlin, Germany) and 2 mM L-
449 glutamine (L-Gln; Biochrom). JAWSII mouse dendritic cells (ATCC CRL-11904) were also
450 purchased from ATCC and cultured in MEM- α (GIBCO BRL, Eggenstein, Germany) supplemented
451 with 20% FBS, 2 mM L-Gln, 1 mM sodium pyruvate (Biochrom), and 5 ng/ml murine GM-CSF
452 (Biotechne, Wiesbaden, Germany). DC2.4 mouse dendritic cells (34) were cultured in RPMI
453 containing 10% FBS, 2 mM L-Gln, 1% non-essential amino acids (Biochrom), 10 mM HEPES (pH
454 7.4), and 50 μ M 2-mercaptoethanol (Sigma-Aldrich, Steinheim, Germany). All cells were cultured
455 at 37°C in a humidified atmosphere containing 6% CO₂ for a maximum of 6 months of culture after
456 thawing of the original stock.

457 **Plasmids**

458 The codon-optimized gene encoding full-length SARS-CoV-2 Spike glycoprotein S of isolate
459 Wuhan-Hu-1 (Genebank accession no. MN908947.1) in plasmids pMA-RQ-SARS2-S flanked with
460 *AatII/MluI* and *NheI/XhoI* restriction sites was obtained by gene synthesis (Invitrogen Life
461 Technology, Regensburg, Germany). The antigen was inserted into plasmids pBRPolIII Δ -MV_{vac2}-
462 GFP(P) or pBRPolIII Δ MV_{vac2}-GFP(H) via *MluI/AatII* to generate pBRPolIII-MV_{vac2}-SARS2-S(P) or
463 pBRPolIII-MV_{vac2}-SARS2-S(H). pBRPolIII Δ -MV_{vac2}-GFP(P) or pBRPolIII Δ MV_{vac2}-GFP(H) were
464 generated by inserting the immediate early CMV promoter sequence from p(+)*PolIII*-MV_{NSe}-GFP(N)
465 (35), which had been modified by site-directed mutagenesis for deleting the *AatII* restriction sites,
466 into pBR-MV_{vac2}-GFP(P) or pBR-MV_{vac2}-GFP(H) (7). For construction of a lentiviral transfer vector
467 encoding SARS-CoV-2 S directly linked to the *egfp* gene as selection marker, the ORF of SARS-
468 CoV-2 S was inserted via *NheI/XhoI* into pCSCW2gluc-IRES-GFP (36) to yield pCSCW2-SARS2-
469 S-IRES-GFP. For construction of a eukaryotic expression plasmid encoding SARS-CoV-2-S, the
470 ORF of SARS2-S was inserted via *NheI/XhoI* into pcDNA3.1(+) (Invitrogen Life Technology) to
471 yield pcDNA3.1-SARS2-S.

472 **Production of lentiviral vectors and generation of antigen-expressing dendritic cell lines**

473 Lentiviral vectors were produced and used for the generation of antigen-expressing dendritic cell
474 lines as described before (7). In short, HIV-1-derived particles pseudotyped with VSV-G were
475 generated using a standard three plasmid system, pMD2.G, pCMV Δ R8.9 (37) with the transfer
476 vector plasmid pCSCW2-SARS2-S-IRES-GFP in combination with PEI transfection of 293T cells

477 (38). Subsequent purification by filtration and ultracentrifugation of supernatants yielded virus
478 stocks were used to transduce murine DC cell lines, DC2.4 and JAWSII, as well as the murine T
479 cell line EL-4, resulting in DC2.4-SARS2-S, JAWSII-SARS2-S, and EL-4_{green}-SARS2-S,
480 respectively, that express the SARS-CoV-2 S protein and GFP and present the respective peptides
481 via MHC-I. Transduced cultures with 1-10% GFP-positive cells were single cell-sorted (BD FACS
482 Aria™ Fusion) for GFP-expressing cells and subsequently characterized for antigen expression.
483 For JAWSII-SARS2-S, the bulk-sorted cells were used in stimulation experiments. For DC2.4-
484 SARS2-S and EL-4_{green}-SARS2-S, clonal cell lines were generated by limiting dilution of bulk-sorted
485 cells and characterized for marker- and antigen-expression.

486 **Viruses**

487 SARS-CoV-2 S-encoding vaccine candidates MeV_{vac2}-SARS2-S(P) or MeV_{vac2}-SARS2-S(H) were
488 generated as described previously (7, 39). Single syncytia were picked and overlaid onto 50%
489 confluent Vero cells cultured in 6-well plates and harvested as “passage 0” (P0) by scraping and
490 freeze-thaw cycle of cells at the time of maximal infection. Subsequent passages were generated
491 after TCID₅₀ titration of infectious virus according to the method of Kaerber and Spaerman (40).
492 Stocks were generated by infection of Vero cells at an MOI = 0.03, and passage 2 (P2) or P3 were
493 used for *in vitro* characterization, while vaccine viruses in P3 or P4 were used for vaccination
494 experiments. Vector control virus MV_{vac2}-ATU(P) (41) was used in P5 for vaccination. SARS-CoV-
495 2 (isolate MUC-IMB1) (kind gift of G. Dobler, Bundeswehr Institute for Microbiology, Germany) was
496 used for SARS-CoV-2 neutralization assays. It was propagated on Vero E6 cells and was titrated
497 via TCID₅₀ as described above for recombinant MeV. All virus stocks were stored in aliquots at -
498 80°C.

499 Multistep viral growth kinetics were analyzed by infecting Vero cells at an MOI of 0.03 in 96-well
500 plates and incubated at 37°C. At various time points, supernatants were clarified by centrifugation,
501 and cells were scraped into OptiMEM and subjected to freeze-thaw cycles. Released and cell-
502 associated viral titers were determined by TCID₅₀ limited dilution method.

503 **Measles virus genome sequence analysis**

504 The RNA genomes of recombinant MeV in P2 or P10 were isolated from infected Vero cells using
505 the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's
506 instructions and resuspended in 50 µL RNase-free water. Viral cDNA was reversely transcribed
507 using Superscript II RT kit (Invitrogen) with 2 µL viral RNA as template and random hexamer
508 primers, according to manufacturer's instructions. For specific amplification of the SARS-CoV-2 S
509 ORF, the respective genomic regions of recombinant MeV were amplified by PCR using primers
510 binding to sequences flanking the regions of interest and the cDNA as template. Detailed

511 description of primers and procedures are available upon request. The PCR products were directly
512 sequenced (Eurofins Genomics, Ebersberg, Germany).

513 **NGS library preparation and sequencing**

514 Total RNA was isolated from Vero cells after 4 days post infection using the Direct-zol RNA isolation
515 kit (Zymo Research). 1 µg of RNA isolate was subjected to rRNA removal with the NEBNext rRNA
516 Depletion Kit (NEB) using the manufacturer's recommendations. The whole 10 µl of the RNA elute
517 was used for reverse transcription with Superscript III (Invitrogen) using the recommended reaction
518 supplemented with 0.5 µl of RiboLock RNase Inhibitor (Thermo Scientific) and 100 pmol of NNSR-
519 RT primer with the following protocol: 45°C 30 min; 70°C, 15 min. The cDNA was bead-purified
520 with 1.8 volume of SPRI Beads (Beckman Coulter), eluted in 27 µl of water and subjected to RNase-
521 H (NEB) digestion at 37°C for 30 min followed by heat inactivation. After bead purification the 20 µl
522 cDNA elute was used for 2nd strand synthesis in a 50 µl reaction containing: 1x NEB Buffer 2, 25
523 nmol dNTP, 5 U of exo(-) Klenow Fragment (NEB), 200 pmol of NNSR-2 Primer for 30 min at
524 37°C. After bead purification half of the DNA elute was used for a 50-µl PCR reaction containing
525 the NEBNext High-Fidelity 2x Master Mix (NEB), 25 pmol, each, of NNSR-Illumina and NNSR-nest-
526 ind primers with the following cycling conditions: 98°C 10 sec; 5 cycles of 98°C 10 sec, 55°C 30
527 sec, 72°C 30 sec; 20 cycles of 98°C 10 sec, 65°C 30 sec, 72°C 30 sec; 72°C 5 min. 15 µl of the
528 PCR reaction was separated on a 1% agarose gel and the smear of 500-700 bp was isolated. The
529 indexed libraries were quantified by qPCR using the NEBNext Library Quant Kit for Illumina (NEB,
530 mixed and sequenced on a MiSeq instrument (Illumina)) with a 2x250 paired-end setup.

531 **RNA sequencing analysis**

532 Quality trimming and adapter removal were performed using fastp (v0.20.0 (42)). Read 1 and 2
533 adapter recognition sequences were provided for adapter removal (Illumina TruSeq Adapter Read
534 1:

535 AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNATCTCGTATGCCGTCTTCTGCT
536 TG, Illumina TruSeq Adapter Read 2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT;
537 NNNNNN: sample-specific index) and the leading two nucleotides were removed from each read
538 (--trim_front1 2 --trim_front2 2). For quality trimming, bases in sliding windows with a mean quality
539 below 30 (-5 -3 --cut_mean_quality 30) were discarded on both sides of the reads. Base correction
540 in overlapping regions (-c) was applied. Reads with Ns and a length below < 30 bp after trimming
541 (-n 0 -l 30) were discarded.

542 Mapping was performed with BWA mem v 0.7.12-r1039 (43), using default parameters unless
543 stated otherwise. Host-derived reads were removed by mapping quality controlled reads against
544 the African green monkey genome (*Chlorocebus sabeus*, RefSeq assembly GCA_000409795.2),
545 specifying the minimum seed length (-k 31). Unmapped reads were extracted using samtools v1.7

546 (44) and bamToFastq v2.17.0 (45), and subsequently mapped to the plasmid reference genomes
547 of either MeV_{vac2}-SARS2-S(H) or MeV_{vac2}-SARS2-S(P), as appropriate. Host-free alignments were
548 deduplicated using picard-tools MarkDuplicates (<http://broadinstitute.github.io/picard>) and left-
549 aligned using GATK LeftAlignIndels v4.0 (46).
550 Sample majority consensus sequences were obtained by substituting minor frequency variants in
551 the respective virus reference sequence for alternative variants with allele frequencies > 50%.
552 Variant calling was performed with LoFreq v2.1.3 (47) using default parameters.

553 **Immunoperoxidase monolayer assay (IPMA)**

554 For immunoperoxidase monolayer assay, Vero cells cultured in flat-bottom 12-well plates were
555 fixed overnight with methanol at -20°C two days after infection with a MOI of 0.01. The fixed cells
556 were then washed three times with 1 mL PBS and subsequently blocked with PBS containing 2%
557 bovine serum albumin (BSA) (Roth, Karlsruhe, Germany) for 30 min at 37°C. The cells were then
558 probed for 1 h with a polyclonal rabbit anti-SARS-CoV-2-S protein antibody (1:2,250; ab252690;
559 Abcam, Cambridge, UK) or a rabbit anti-MeV N protein antibody (1:1,000, ab23974, Abcam) in
560 PBS with 2% BSA. The cells were washed 3 times with 1 ml PBS and subsequently incubated with
561 the secondary HRP-coupled donkey anti-rabbit IgG(H+L) polyclonal antibody (1:1,000; 611-7202;
562 Rockland, Gilbertsville, USA) for 1 h at 37°C. Then, the cells were washed 3 times, again. For
563 detection, the cells were stained with TrueBlue peroxidase substrate solution (SeraCare, Milford,
564 USA).

565 **Western Blot Analysis**

566 Cells were lysed and immunoblotted as previously described (48). Rabbit anti-SARS-S protein
567 antibody (1:3,000; ab252690; Abcam), rabbit anti-MeV-N protein polyclonal antibody (1:5,000;
568 ab23974; Abcam), and a mouse anti-β-actin antibody (1:5,000; ab6276; Abcam) were used.
569 Donkey anti-rabbit IgG-HRP (H&L) polyclonal antibody (1:10,000; 611-7202; Rockland) and goat
570 anti-mouse IgG-HRP (1:10,000; A2554-1ML; Merck, Darmstadt, Germany) served as secondary
571 antibodies. Peroxidase activity was visualized with an enhanced chemiluminescence detection kit
572 (Thermo Scientific, Bremen, Germany) on ChemiDoc MP Imaging System (Biorad, Dreieich,
573 Germany).

574 **Animal experiments**

575 All animal experiments were carried out in compliance with the regulations of German animal
576 protection laws and as authorized by the RP Darmstadt in consideration of the ARRIVE guidelines.
577 Six- to 12-week-old old, treatment-naive IFNAR^{-/-}-CD46Ge mice (10) that are deficient for type I
578 IFN receptor and transgenically express human CD46 were bred in-house under SPF conditions
579 and regularly controlled by animal care takers and institutional veterinarians for general signs of

580 well-being, and animal weight was additionally controlled once a week during the experiments. For
581 the experiments, animals were randomized for age- and sex-matched groups and housed in IVC
582 cages in groups of 3 to 5 animals with nest packs as environmental enrichment at room temperature
583 with regular 12 h day and night intervals. Group sizes were calculated based on statistical
584 considerations to yield sufficient statistical power as authorized by the respective competent
585 authority. These animals were inoculated intraperitoneally (i.p.) with 1×10^5 TCID₅₀ of recombinant
586 vaccine viruses in 200 μ l volume, or subcutaneously (s.c.) with 10 μ g recombinant SARS-CoV-2 S
587 protein (Sino Biological Europe, Eschborn, Germany) adjuvanted with 500 μ g aluminium hydroxide
588 (Alhydrogel adjuvant 2%, vac-alu-250, InvivoGen, San Diego, CA, USA) in 100 μ l volume on days
589 0 and 28. 200 μ l blood was collected on days 0, and 28, while final serum was collected on day 49
590 post initial immunization (p.i.). serum samples were stored at -20°C. Mice were euthanized on day
591 49 p.i., and splenocytes were harvested for assessment of cellular immune responses.

592 **Total IgG and IgG1/IgG2a quantification**

593 MeV bulk antigens (10 μ g/mL; Virion Serion, Würzburg) or recombinant SARS-CoV-2 S protein (5
594 μ g/mL) were coated in 50 μ l carbonate buffer (Na₂CO₃ 30 mM; NaHCO₃ 70 mM; pH 9.6) per well
595 on Nunc Maxisorp® 96 well ELISA plates (ebioscience) and incubated overnight at 4°C. The plates
596 were washed three times with 200 μ l ELISA washing buffer (PBS, 0.1% Tween 20 (w/v)) and
597 blocked with 100 μ L Blocking buffer (PBS; 5% BSA; 0.1% Tween 20) for at least 2 h at room
598 temperature. Mouse sera were 5-fold serially diluted in ELISA dilution buffer (PBS, 1% BSA, 0.1%
599 Tween 20), and 50 μ L/well were used for the assay. The plates were incubated at 37°C for 2 h and
600 washed three times with ELISA washing buffer, followed by incubation with 50 μ l/well of HRP-
601 conjugated rabbit anti-mouse total IgG (1:1,000 in ELISA dilution buffer; P0260, Dako Agilent,
602 Santa Clara, CA, USA), goat-anti-mouse IgG1 (1:8,000 in ELISA dilution buffer; ab97240, Abcam,
603 Cambridge, UK), or goat-anti-mouse IgG2a (1:8,000 in ELISA dilution buffer; ab97245, Abcam) at
604 room temperature for 1 h. Subsequently, the plates were washed four times and 100 μ L TMB
605 substrate (ebioscience) was added per well. The reaction was stopped by addition of 50 μ L/well
606 H₂SO₄ (1 N) and the absorbance at 450 nm (specific signal) and 630nm (reference wavelength)
607 was measured.

608 **Th1/Th2 cytokine multiplex assay**

609 Quantification of Th1/Th2 cytokines in supernatant of splenocytes was performed using mouse
610 high sensitivity T cell magnetic bead panel assay (MHSTCMAG-70K, Merck, Darmstadt, Germany).
611 5×10^5 isolated splenocytes were co-cultured with different stimuli in 200 μ L RPMI + 10% FBS, 2
612 mM L-Gln, and 1% penicillin-streptomycin for 36 h. For re-stimulation of SARS-CoV-2 S protein-
613 specific T cells, splenocytes were co-cultivated with 5×10^4 DC2.4 dendritic cells, the corresponding

614 cell line transgenically expressing SARS-CoV-2 S protein or medium alone. After 36 h, cells were
615 spun down and supernatants were collected and stored at -20°C till assayed. For multiplex assay,
616 cytokines were coupled over night to magnetic beads coated with capture antibodies, labeled with
617 biotinylated detection antibody and incubated with Streptavidin-PE conjugate. Fluorescence was
618 measured using MAGPIX with xPONENT software (Luminex Instruments, Thermo Scientific,
619 Bremen, Germany).

620 **Neutralization Assay**

621 Virus neutralizing titers (VNT) were quantified as described previously (7). Towards this, sera were
622 serially diluted in 2-fold dilution steps in DMEM in duplicates. A total of 50 PFU of MV_{vac2}-GFP(P)
623 or 100 TCID₅₀ of SARS-CoV-2 (isolate MUC-IMB1) were mixed with diluted sera and incubated at
624 37°C for 1 h. MeV or SARS-CoV-2 virus-serum suspensions were added to 1×10⁴ Vero or Vero E6
625 cells, respectively, seeded 4 h prior to the assay in 96-well plates and incubated for 4 days at 37°C.
626 VNTs were calculated as the reciprocal of the highest mean dilution that abolished infection.

627 **IFN-γ ELISpot Analysis**

628 Murine interferon gamma (IFN-γ) enzyme-linked immunosorbent spot (ELISpot) assays were
629 performed using the Mouse IFN-γ ELISPOT Pair kit including capture and detection antibody (BD
630 Bioscience, Franklin Lakes, NJ, USA) and HRP Streptavidin (BD Bioscience) for ELISpot detection
631 in combination with multiscreen immunoprecipitation (IP) ELISpot polyvinylidene difluoride (PVDF)
632 96-well plates (Merck Millipore, Darmstadt, Germany) according to the manufacturer's instructions.
633 5×10⁵ isolated splenocytes were co-cultured with different stimuli in 200 μL RPMI + 10% FBS, 2
634 mM L-Gln, and 1% penicillin-streptomycin for 36 h. For re-stimulation of SARS-CoV-2 S protein-
635 specific T cells, splenocytes were co-cultivated with 5×10⁴ JAWSII, DC2.4 dendritic cells, or the
636 corresponding cell lines transgenically expressing SARS-CoV-2 S protein. In parallel, splenocytes
637 were stimulated with 10 μg/mL MeV bulk antigen (Virion Serion). For general T cell stimulation, 10
638 μg/mL concanavalin A (ConA, Sigma-Aldrich) was used, and as negative control, splenocytes were
639 left untreated. After 36 h, cells were spun down, supernatants were removed, and cells were lysed
640 in the wells by hypotonic shock. Plates were incubated with biotin-conjugated anti-IFN-γ detection
641 antibodies and streptavidin-HRP according to the manufacturer's instructions. 3-Amino-9-ethyl-
642 carbazole (AEC; Sigma-Aldrich) was dissolved in N,N-dimethylformamide (Merck Millipore) and
643 used for peroxidase-dependent staining. Spots were counted using an Eli.Scan ELISpot scanner
644 (AE.L.VIS, Hamburg, Germany) and ELISpot analysis software Eli.Analyse V5.0 (AE.L.VIS).

645 **Intracellular cytokine staining**

646 For flow cytometry-based analysis of cytokine expression by intracellular cytokine staining (ICS),
647 splenocytes of vaccinated mice were isolated, and 2×10⁶ splenocytes per mouse were cultivated

648 in 200 μ L RPMI1640 + 10% FBS, 2 mM L-Gln, 1 \times non-essential amino acids (Biochrom), 10 mM
649 HEPES, 1% penicillin-streptomycin, 50 μ M β -mercaptoethanol, 10 μ g/mL brefeldin A (Sigma-
650 Aldrich) with DC2.4-SARS2-S cells as used for ELISpot analysis. For general T cell stimulation,
651 0.25 μ g/mL tetradecanoylphorbol acetate (TPA, Sigma Aldrich) and 0.5 μ g/mL ionomycin (Iono,
652 Sigma-Aldrich) were used as positive control, and medium alone served as negative control.
653 Splenocytes were stimulated for 5 h at 37°C. Subsequently, cells were stained with fixable viability
654 dye eFluor450 (eBioscience), α -CD4-PE (1:2,000; Cat.-No. 553049 BD, Franklin Lakes, NJ, USA),
655 α -CD8-FITC (1:500; Cat.-No. 553031, BD), and α -CD3-PerCPCy5.5 (1:500; Cat.-No. 550763, BD).
656 Subsequent to permeabilization with Fixation/Permeabilization Solution (BD) and Perm/Wash
657 Buffer (BD), cells were stained with α -IFN- γ -APC (1:500; Cat.-No. 554413, BD), α -IL-2-
658 AlexaFluor700 (1:200; Cat.-No. 503818, Biolegend, San Diego, USA) and α -TNF- α -Pe-Cy7 (1:500;
659 Cat.-No. 557644, BD). Cells were fixed with ice-cold 1% paraformaldehyde (PFA) in PBS and
660 analyzed via flow cytometry using an LSR II SORP flow cytometer (BD) and DIVA software (BD).

661 **T cell proliferation assay**

662 Splenocytes isolated three weeks after the second immunization were labeled with 0.5 μ M
663 carboxyfluorescein-succinimidyl-ester (CFSE) (ebioscience, Life Technologies, Carlsbad, CA,
664 USA) as previously described (49). In brief, 5 \times 10⁵ labelled cells were seeded in RPMI 1640
665 supplemented with 10% mouse serum, 2 mM L-Glutamine, 10 mM HEPES, 1%
666 penicillin/streptomycin, and 100 μ M 2-mercaptoethanol in 96-wells. 200 μ L Medium containing 10
667 μ g/ml Concanavalin A (Con A, Sigma-Aldrich), 10 μ g/ml MeV bulk antigen (Virion Serion), or 5 \times 10³
668 DC2.4-SARS2-S cells were added to each well, and cultured for 6 days. Medium and wild type
669 DC2.4 and JAWSII cells served as controls. Stimulated cells were subsequently stained with CD3-
670 PacBlue (1:50; clone 500A2; Invitrogen Life Technologies), CD8-APC (1:100; clone 53-6.7;
671 ebioscience) and CD4-PE (1:2000; Cat. 553049; BD) antibodies and fixed with 1% PFA in PBS.
672 Finally, the stained cells were analyzed by flow cytometry using an LSR II flow cytometer (BD) and
673 FCS Express software (De Novo Software).

674 **CTL killing assay**

675 For re-stimulation of T cells isolated 3 weeks after the second immunization, 5 \times 10⁶ splenocytes
676 were co-cultured with 5 \times 10⁴ DC2.4-SARS2-S cells for 6 days in 12-wells in RPMI 1640
677 supplemented with 10% FBS, 2 mM L-Glutamin, 1 mM HEPES, 1% penicillin/streptomycin, 100 μ M
678 2-mercaptoethanol, and 100 U/ml murine rIL-2 (Peprotech, Hamburg, Germany). 5 \times 10³ EL-4_{red}
679 cells were labeled with 0.5 μ M CFSE and mixed with 5 \times 10³ EL-4_{green}-SARS2-S cells per well.
680 Splenocytes were counted and co-cultured with EL-4 target cells at the indicated ratios for 4 h at
681 37°C. Afterwards, EL-4 cells were labeled with Fixable Viability Dye eFluor® 780 (ebioscience),

682 fixed with 1% paraformaldehyde (PFA), and analyzed by flow cytometry using an LSR II flow
683 cytometer (BD) and FCS Express. For indication of Antigen:NC EL-4 ratio the cell count of viable
684 SARS-CoV-2 S-expressing cells was divided by the population of viable negative controls.

685 **Statistical analyses**

686 To compare the means of different groups in growth kinetics, a non-parametric One-way ANOVA
687 was performed. For ICS analysis, the non-parametric two-tailed Mann-Whitney test was used to
688 compare cytokines levels between DC2.4 and DC2.4-SARS2-S- restimulated splenocytes within
689 the MeV_{-vac2}-SARS-2-S(H) vaccine group. Note, that these exploratory analyses have been done
690 without correction for multiple testing. For proliferation assay the mean differences were calculated
691 and analyzed using one-tailed Mann-Whitney t-test. To all three groups in CTL killing assays a
692 linear curve was fitted for antigen vs. logarithmised effector-target ratio E:T. The p values testing
693 for differences in slopes were calculated and populations of SARS2-S(H) compared with control
694 ATU vaccinated cells. The p values were not adjusted for multiplicity due to the explorative
695 character of the study. For VNT and fusion activity statistical analysis, one-way ANOVA was
696 performed in combination with Tukey's Multi comparison test to compare all pair means. For
697 multiplex statistical analysis, two-way ANOVA analysis was applied with paired Tukey's Multi
698 comparison test as post hoc test. For statistical analysis of grouped ELISpot data, two-way ANOVA
699 analysis was applied with paired Tukey's Multi comparison test.

700

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702

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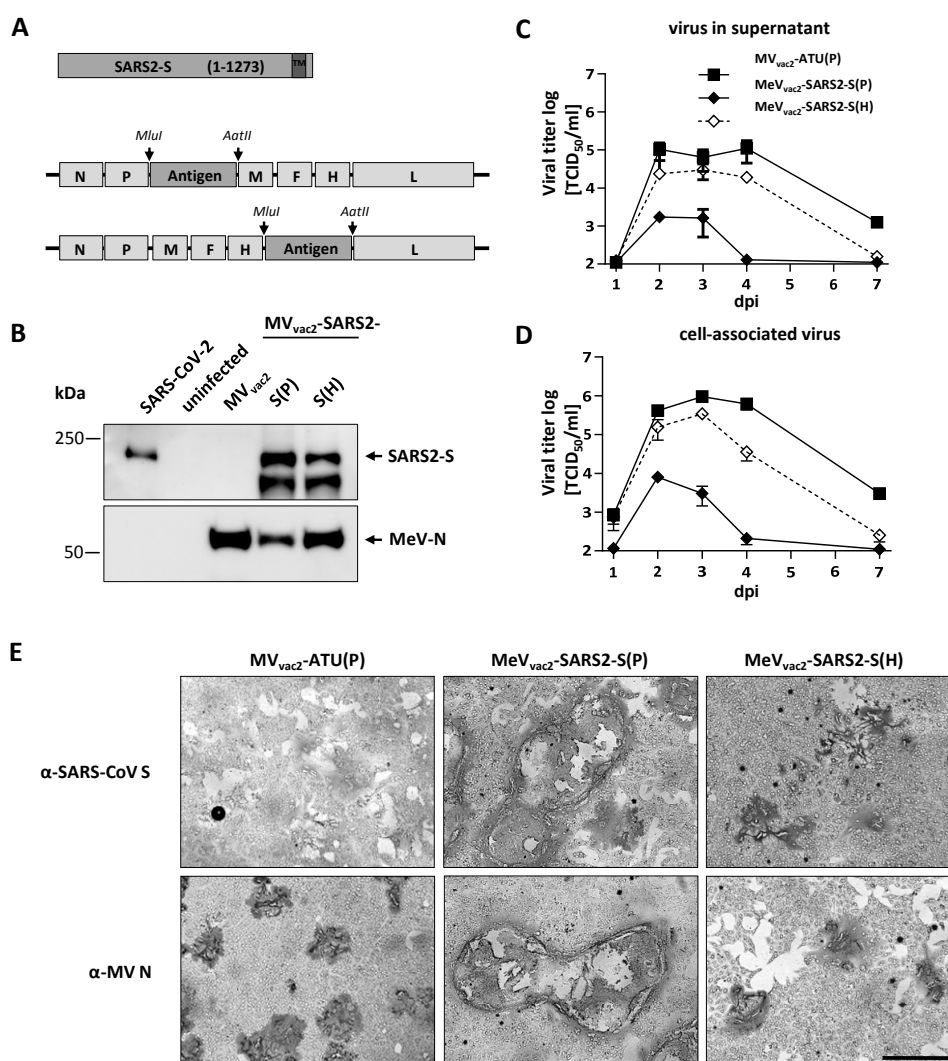
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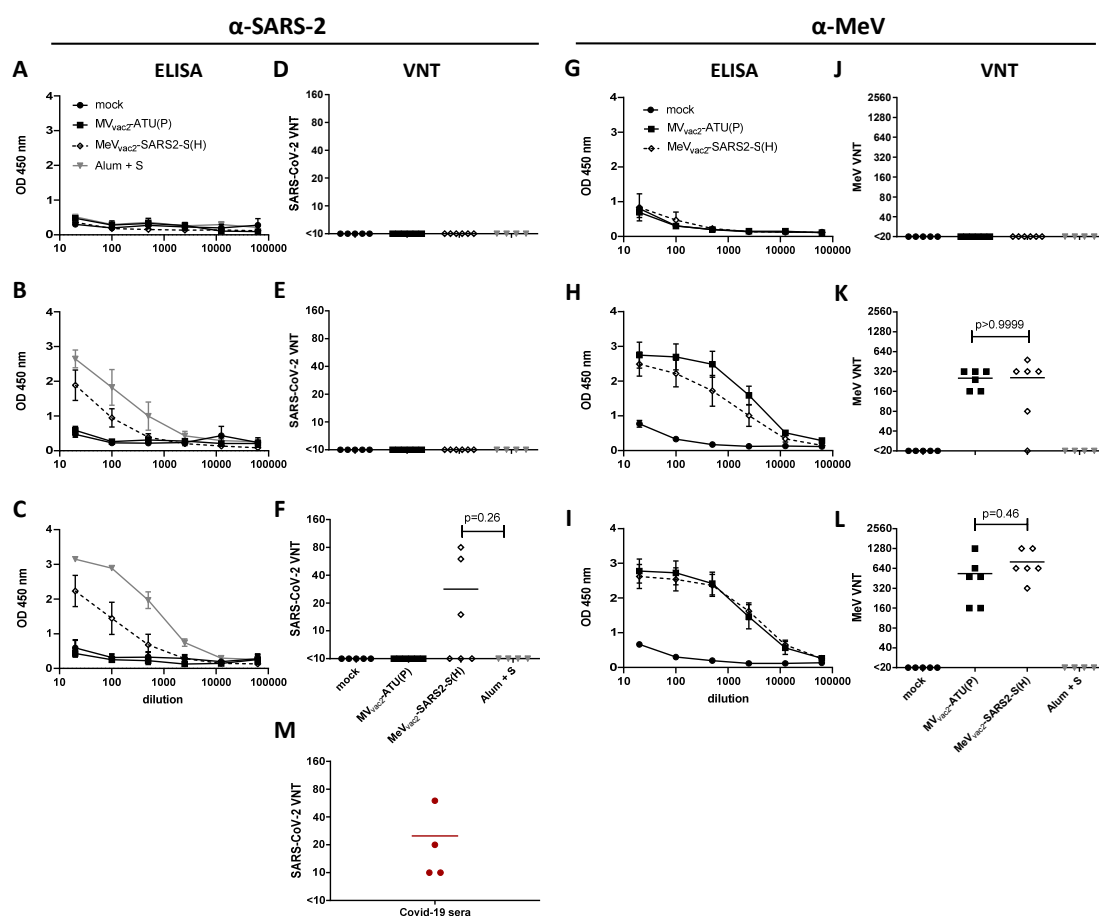
891 **Figures**



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894 **Fig. 1: Generation and *in vitro* characterization of MeV_{vac2}-SARS2-S(P) and MeV_{vac2}-SARS2-**
 895 **S(H).** (A) Schematic depiction of full-length SARS-CoV-2 S and recombinant MeV_{vac2} genomes
 896 used for expression of this antigen (lower schemes). Antigen or antigen encoding genes are
 897 depicted in dark grey; MeV viral gene cassettes (in light grey) are annotated. *MluI* and *AatII*
 898 restriction sites used for cloning of antigen-genes into post P or post H ATU are highlighted (B)
 899 Immunoblot analysis of Vero cells infected at an MOI of 0.01 with MeV_{vac2}-SARS2-S(P), MeV_{vac2}-
 900 SARS2-S(H), or MV_{vac2}-ATU(P) (MV_{vac2}) as depicted above lanes. Uninfected cells served as mock.
 901 Blots were probed using rabbit polyclonal anti-SARS spike antibody (upper blot) or mAb reactive
 902 against MeV-N (lower blot). Arrows indicate specific bands. (C, D) Growth kinetics of recombinant
 903 MeV on Vero cells infected at an MOI of 0.03 with MV_{vac2}-ATU(P) or MeV_{vac2}-SARS2-S encoding
 904 extra genes in post H or post P. Titers of samples prepared at indicated time points post infection
 905 were titrated on Vero cells. Means and standard deviations of three to five independent experiments

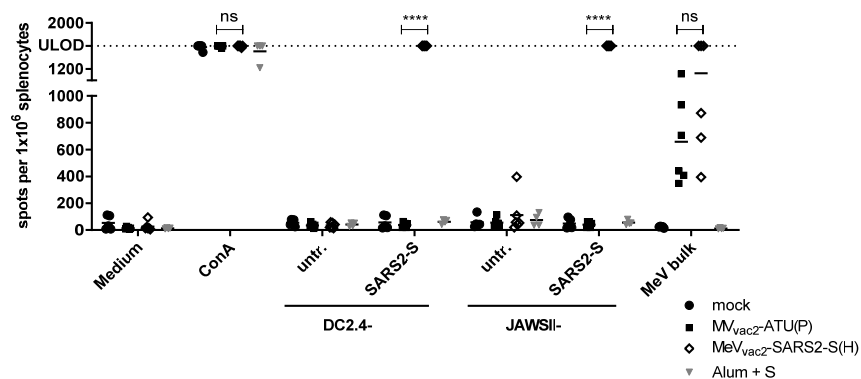
906 are presented. (E) SARS-CoV-2 S protein expression in Vero cells was verified via
907 immunoperoxidase monolayer assay. 50× magnification; scale bar, 500 μm.



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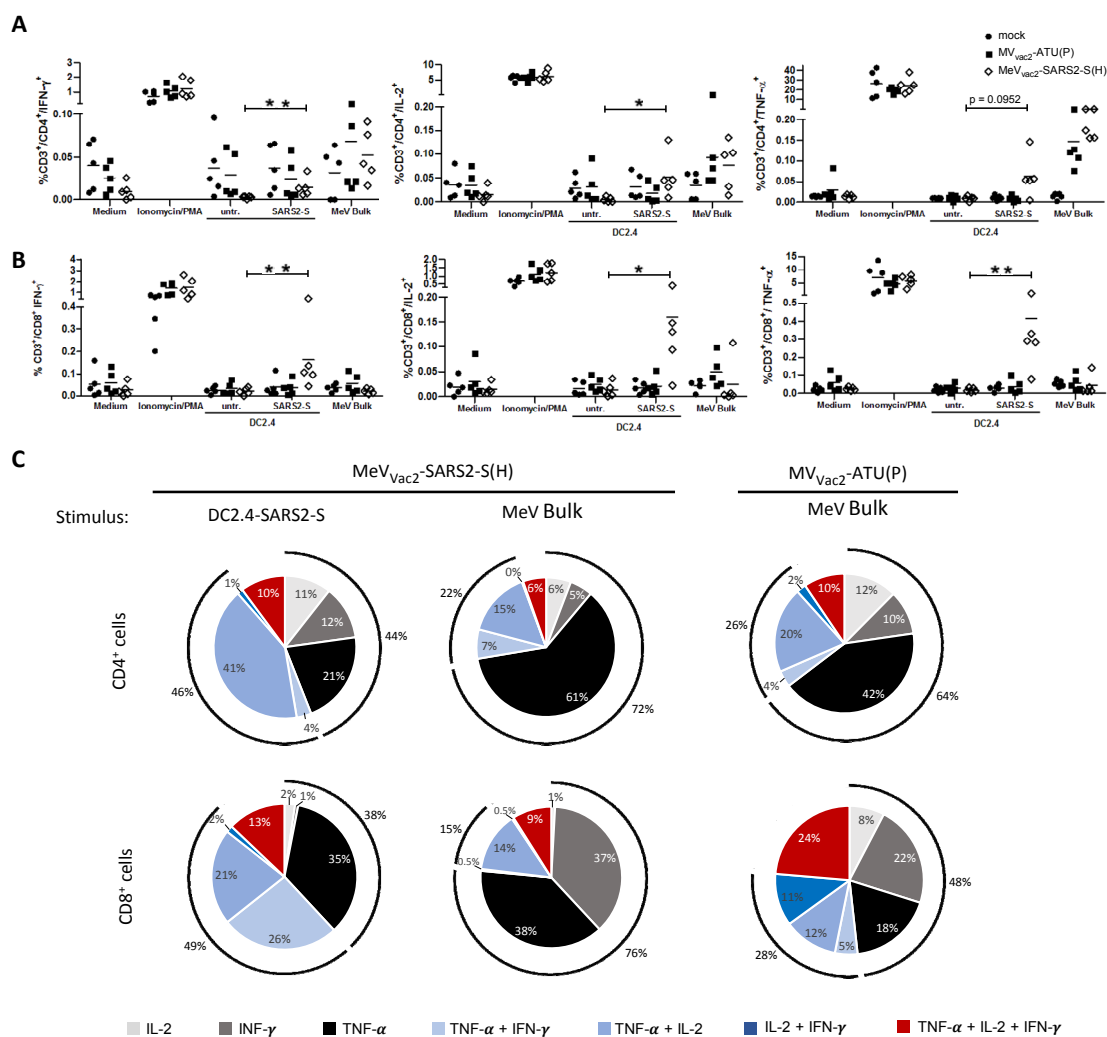
909 **Fig. 2:** Induction of a-SARS-CoV-2 S and a-MeV specific antibodies. Sera of mice vaccinated on
 910 days 0 and 28 with indicated viruses or Alum-adjuvanted S protein were sampled on day 0 (A, D,
 911 E, F), day 28 after prime- (B, E, H, K) and day 49 after boost-immunization (C, F, I, L) and analyzed
 912 for antibodies specific for SARS-CoV-2 S or MeV. Medium-inoculated mice served as mock. Pan-
 913 IgG binding to recombinant SARS-CoV S (A – C) or MeV bulk antigens (G – I) were determined by
 914 ELISA via the specific OD 450 nm value. Depicted are means and respective standard deviation of
 915 the mean (SEM) of each group (n = 5 - 6). Virus neutralizing titers (VNT) in vaccinated mice for
 916 SARS-CoV-2 (D - F) or MeV (J – L) were calculated as reciprocal of the highest dilution abolishing
 917 infectivity. (M) SARS-CoV-2 VNT of 4 human Covid-19 convalescent sera. Dots represent single
 918 individuals; horizontal line represents mean per group. For statistical analysis of VNT data, one-
 919 way ANOVA was performed in combination with Tukey's Multi comparison test to compare all pair
 920 means.

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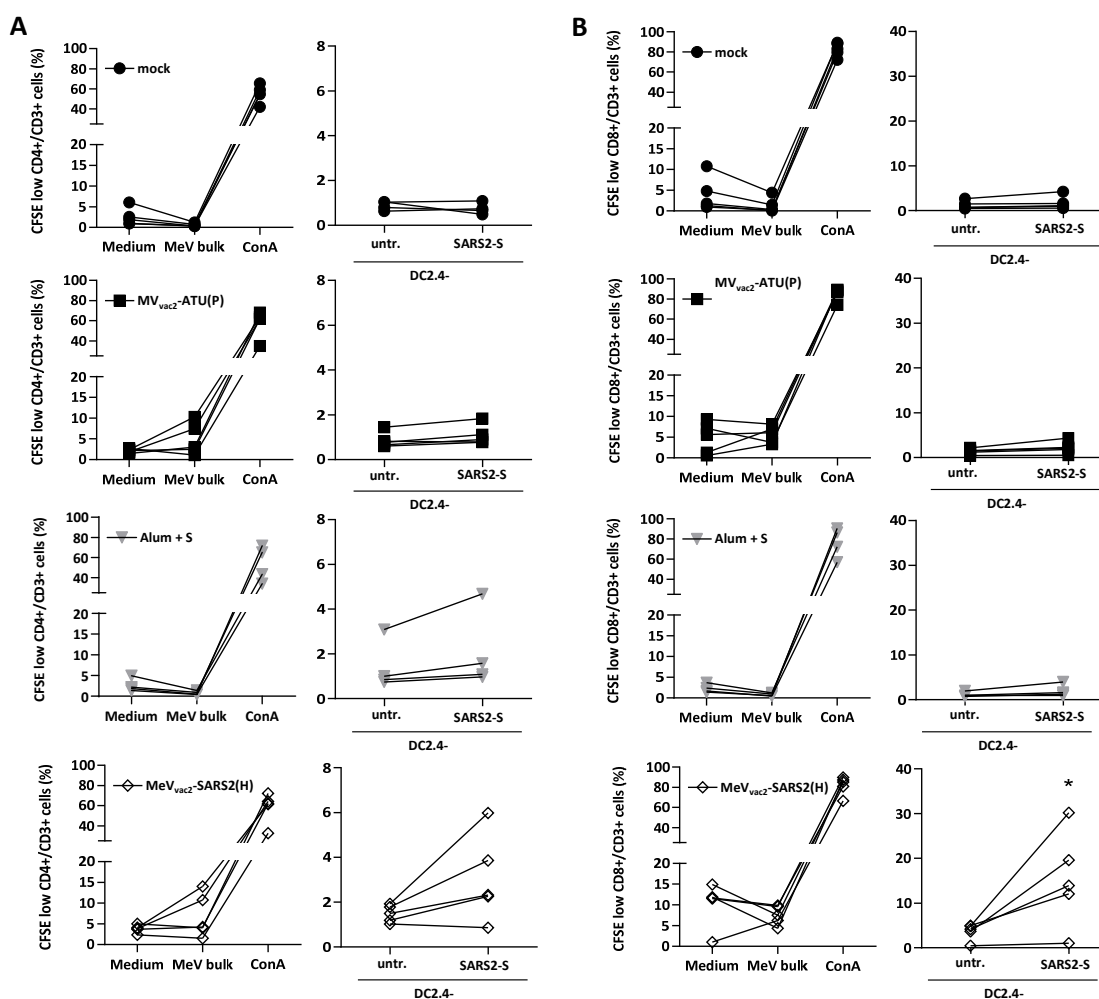
923 **Fig. 3: Secretion of IFN- γ after antigen-specific re-stimulation of splenocytes.** IFN- γ ELISpot
 924 analysis using splenocytes of mice vaccinated on days 0 and 28 with indicated vaccines, isolated
 925 21 days after boost immunization, and after co-culture with DC2.4 or JAWSII dendritic cell lines
 926 transgenic for SARS-CoV-2 S (SARS2-S) or untransduced controls (untr.). To analyze cellular
 927 responses directed against MeV, splenocytes were stimulated with 10 μ g/mL MeV bulk antigens or
 928 were left unstimulated as controls (medium). The reactivity of splenocytes was confirmed by
 929 Concanavalin A (ConA) treatment (10 μ g/mL). The number of cells per 1 \times 10⁶ splenocytes represent
 930 the amount of cells expressing IFN- γ upon re-stimulation. Dots represent individual animals,
 931 horizontal bars mean per group (n = 5 - 6). Samples above the upper detection limit (ULOD) were
 932 displayed as such. For statistical analysis of grouped ELISpot data, two-way ANOVA analysis was
 933 applied with paired Tukey's Multi comparison test used as post hoc test. ns, not significant (p > 0.05);
 934 ****, p < 0.0001.



935
 936 **Fig. 4: Detection of multi-functional T-cell responses induced by vaccination with MeV_{vac2}-**
 937 **SARS2-S(H).** Harvested splenocytes of MeV_{vac2}-SARS2-S(H) vaccinated mice (same as depicted
 938 in Fig. 3) were re-stimulated and subjected to intracellular staining (ICS) for IFN- γ , TNF- α , and IL-
 939 2, and stained for extracellular T-cell markers CD3, CD4, and CD8 for flow cytometry analysis.
 940 Quantification of flow cytometry data of (A) CD4⁺ and (D) CD8⁺- positive T cells after co-culture
 941 with antigen-presenting DC2.4-SARS2-S or parental DC2.4 control cells, or after incubation with
 942 indicated stimuli (MeV bulk antigen (MeV bulk), or untreated cells (mock); reactivity of splenocytes
 943 was confirmed by ionomycin and phorbol myristate acetate (PMA) treatment (10 μ g/mL). Dots
 944 represent individual animals, horizontal bars mean. Mann-Whitney test was used to compare
 945 cytokines levels between DC2.4 and DC2.4-SARS2-S re-stimulated splenocytes in the MeV_{vac2}-
 946 SARS2-S(H) vaccine group without correction for multiple testing because of the exploratory
 947 character of the study. *, p<0.05; **, p<0.01. (C) reveals poly-functional T cells depicted in the pie-
 948 chart as fractions of cell populations expressing one, two, or all three of the tested cytokines and
 949 indicating the size of each fraction among all responsive T cells.

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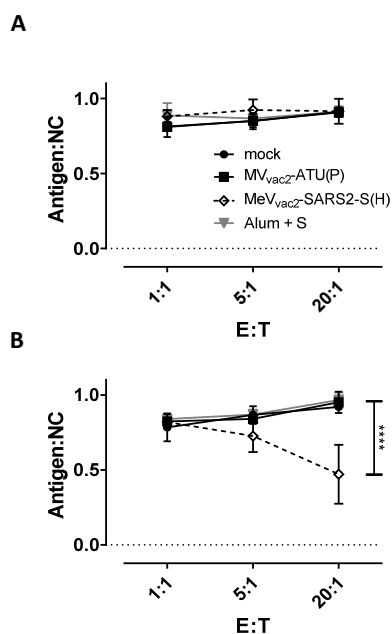


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954 **Fig. 5: Ag-specific proliferation of SARS-CoV-2 S-specific T cells.** Proliferation assay using
 955 splenocytes of mice vaccinated on days 0 and 28 with indicated viruses, isolated 21 days after
 956 boost immunization, after co-culture with DC2.4 dendritic cell line transgenic for SARS-CoV-2 S
 (SARS2-S) or untransduced parental DC2.4 (untr.). Depicted are the percentages of (A) CD4⁺
 957 or (B) CD8⁺ T cells with low CFSE staining, indicating proliferation in the samples. To analyze cellular
 958 α -MeV responses, splenocytes were stimulated with 10 μ g/ml MeV bulk antigens or were left
 959 unstimulated (medium). The reactivity of splenocytes was confirmed by concanavalin A (ConA)
 960 treatment (10 μ g/ml). Results for splenocytes of vaccinated mice are displayed individually and the
 961 trend between paired unstimulated and re-stimulated samples is outlined (n = 2-4). One-tailed
 962 Mann-Whitney t-test. *, p<0.05.

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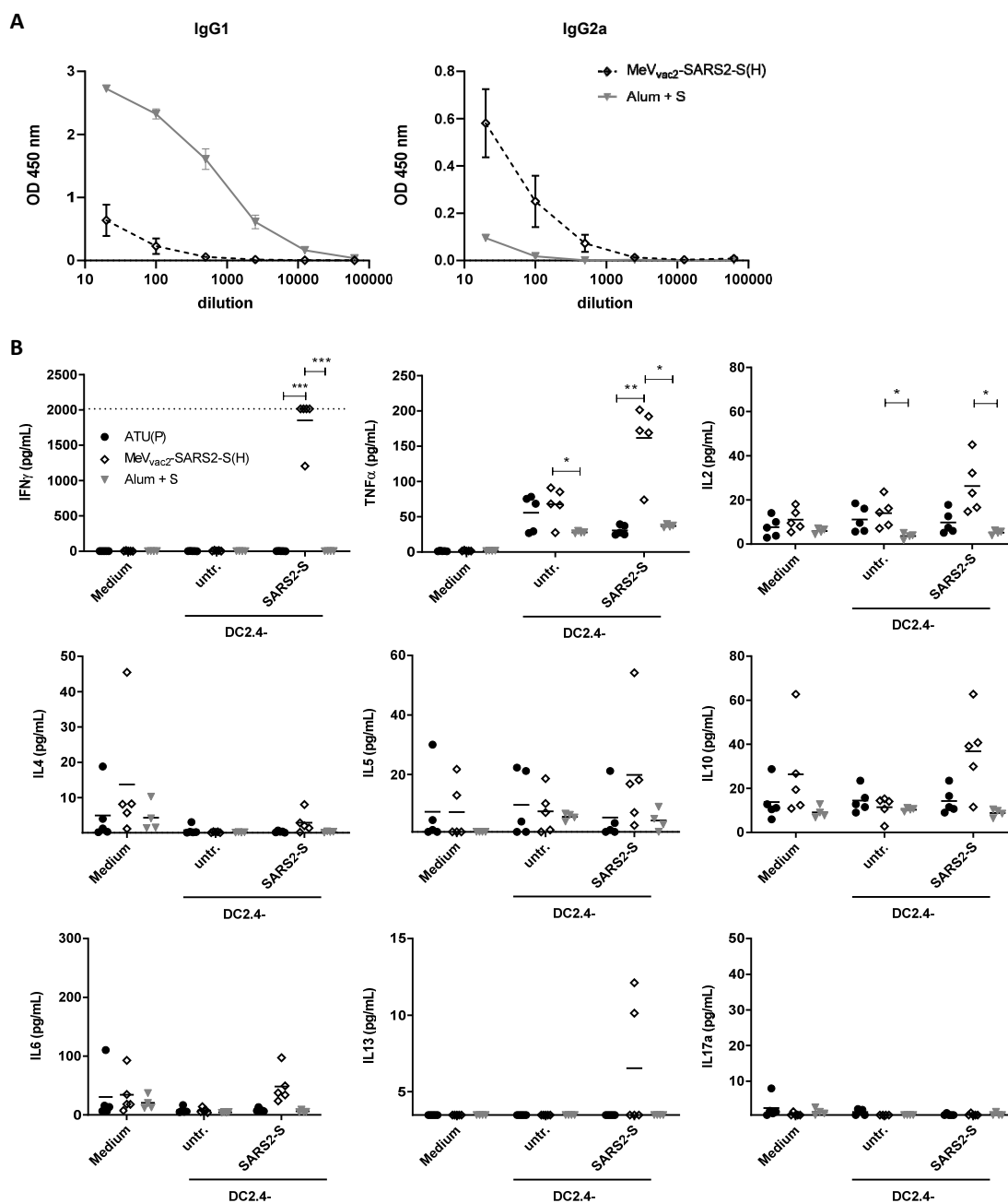


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Fig. 6: Antigen-specific killing activity of SARS-CoV-2 S-specific T cells. Killing assay using
965 splenocytes of mice vaccinated on days 0 and 28 isolated 21 days after the second immunization.
966 Splenocytes were co-cultured with DC2.4 (A) or with antigen-presenting DC2.4-SARS2-S (B)
967 or for 6 days. Activated CTLs were then co-cultured with EL-4_{green}-SARS2-S target cells (Antigen)
968 and EL-4_{red} control cells (NC) at indicated E:T ratios for 4 h. Ratio of living target to non-target cells
969 (Antigen:NC) was determined by flow cytometry. Depicted are means and standard deviation
970 of each group (open diamonds, MeV_{vac2}-SARS2-S(H); filled circles, mock; filled squares, MV_{vac2}-
971 ATU(P); grey triangles: S protein + Alum) (n = 3 - 5). For statistical analysis of grouped ELISpot
972 data, two-way ANOVA analysis was applied with paired Tukey's Multi comparison test used as post
973 hoc test. ****, p<0.0001.

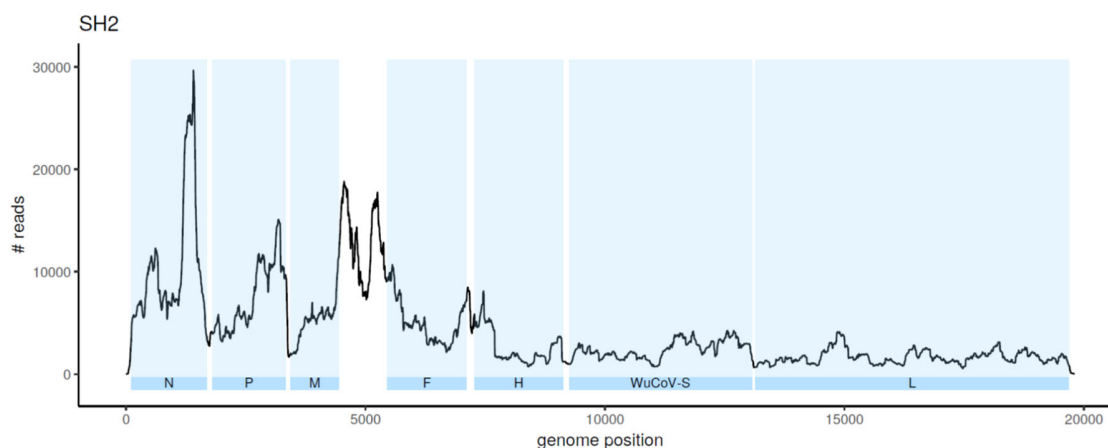
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 976 **Figure 7: Immune bias of induced responses.** To analyze skewing of immune responses
 977 towards Th1- or Th2-biased immunity (A) sera and (B) splenocytes of vaccinated mice depicted
 978 before were analyzed. (A) Sera of mice vaccinated on days 0 and 28 with MeV_{vac2}-SARS2-S(H) or
 979 Alum-adjuvanted S protein already shown in Fig. 2 were analysed for IgG1- or IgG2a-type
 980 antibodies specific for SARS-CoV-2 S. IgG1 (left panel) or IgG2a (right panel) binding to
 981 recombinant SARS-CoV S were determined by ELISA via the specific OD 450 nm value. Depicted
 982 are means and respective standard deviation of the mean (SEM) of each group (n = 5 - 6). (B)
 983 Splenocytes of the same mice already shown in Figs. 3 to 6 were analysed by multiplex cytokine
 984 analysis for secretion of typical marker cytokines in the supernatant after re-stimulation by co-

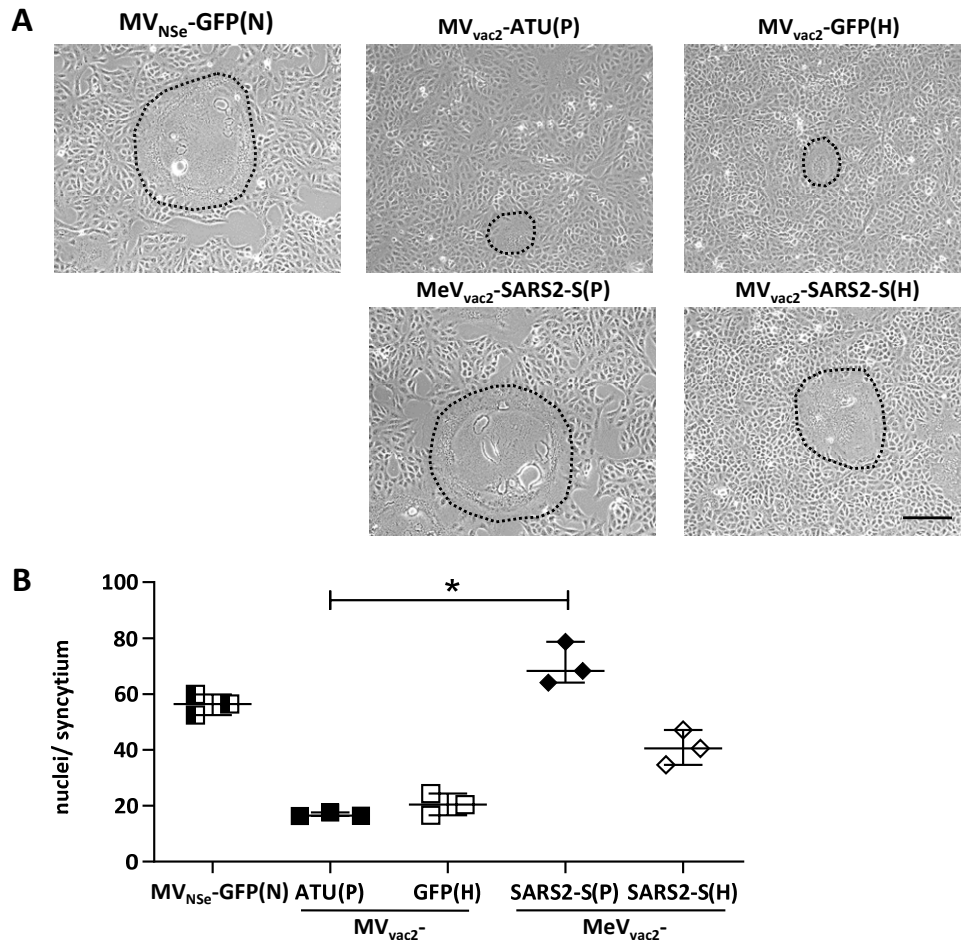
985 culture with antigen-presenting DC2.4-SARS2-S cells. DC2.4 cells served as non-specific control
986 stimulus. Dots represent individual animals, horizontal bars mean per group (n = 4 - 5). IFN- γ : upper
987 limit of detection (ULOD): 2015.2 pg/mL; IL-6: ULOD: 3992,4 pg/mL; IL-17a lower limit of detection
988 (LLOD): 0.473 pg/mL; IL-4 LLOD: 0.095 pg/mL; IL-5 LLOD: 0.685 pg/mL; IL-13 LLOD: 3.463 pg/mL.
989 For statistical analysis of grouped multiplex data, two-way ANOVA analysis was applied with paired
990 Tukey's Multi comparison test as post hoc test. *, p<0.05; **, p<0.01; ***, p<0.00
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992 **Supplementary Figures**
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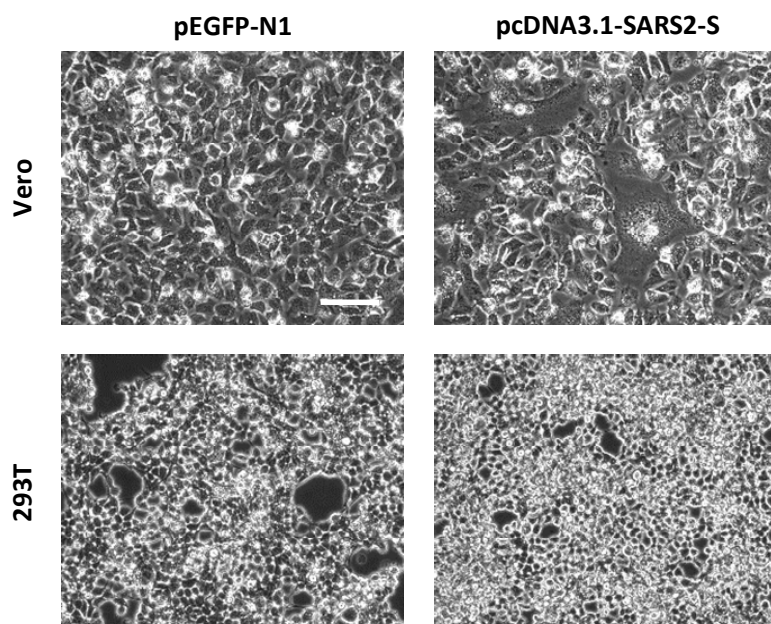
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Suppl. Fig. S1: Coverage of of vaccine candidate MeV_{vac2}-SARS2-S(H) genome during next generation sequencing. Schematic depiction of read frequency at each position of the vaccine virus genome. Blue areas indicate respective viral coding sequences, white areas indicate intergenic regions and untranscribed terminal regions (UTRs) of the genome. Coverage across the genome was sufficient for variant detection and reflects the transcription gradient typically observed in measles virus total RNAseq data. Since the majority of reads are mRNA-derived, low read numbers decrease strongly between the coding regions and continually towards the 5' end.



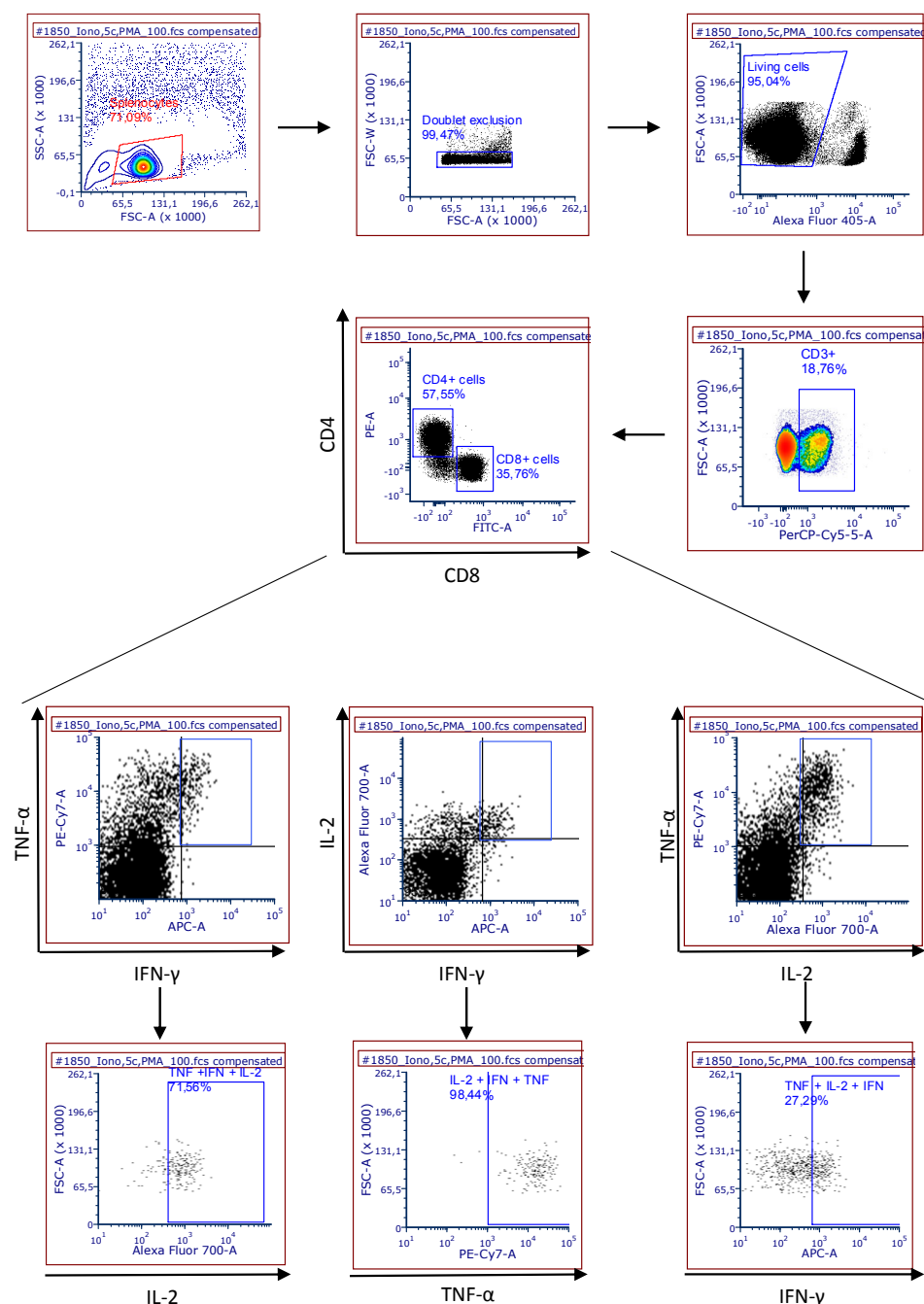
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Suppl. Fig. S2: Characterization of fusogenic phenotype of MeV_{vac2}-SARS2-S(P) and MeV_{vac2}-SARS2-S(H). (A) Photographs of fusion activity of Vero cells infected at an MOI of 0.01 with MeV_{vac2}-SARS2-S(P) or MeV_{vac2}-SARS2-S(H) encoding SARS-CoV-2 S in additional transcription units post P or post H, respectively, in direct comparison to MV_{vac2}-ATU(P) or MV_{vac2}-GFP(H) control vaccine viruses or MV_{NSe}-GFP(N) hyperfusogenic oncolytic MeV. Representative picture of one out of three independent experiments. Scale bar represents 200 μm. (B) Cell fusion was quantified 30 h after infection. For statistical analysis, one-way ANOVA was performed in combination with Tukey's Multi comparison test to compare all pair means. *, p<0.05.



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Suppl. Fig. S3: Expression of SARS-CoV-2 S protein in Vero and 293T cells. Photographic depiction of fusion activity in Vero or 293T cells 48 h after transfection with 1 μ g of SARS-CoV-2 S expression plasmid of control DNA. One representative out of three independent experiments is shown. Scale bar represents 100 nm.



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Suppl. Fig. S4: Gating strategy for intracellular cytokine staining. Exemplary depiction of the gating strategy to analyze T cells after re-stimulation and staining for cytokine induction. The gating strategy includes cell doublet exclusion, selection for living cells and separation of CD8+ and CD4+ T cells within CD3+ splenocyte populations. Respectively gated T cell populations were then analysed for expression of IFN-g, TNF-a, or IL-2. Multi-colour flow cytometry allows assessment of double- or triple-positive cells, exemplarily shown for CD4+ T cells after stimulation with ionomycin and PMA.